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Study of molecular interactions between Chitosan and Vi Antigen

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ABSTRACT

Chitosan has attracted much interest due to its special physical and chemical properties related to drug administration. Nanoparticles delivery systems from Vi Antigen are a promising approach in the struggle against typhoid fever. In this paper, we reported the obtainment and the characterization of Vi Antigen by Infrared spectroscopy as well as Molecular Modeling and Computational Chemistry studies of the Chitosan-Vi Antigen interaction through theoretical models. The results of the theoretical and experimental Infrared spectroscopy showed important bands related to *N*-Acetyl and *O*-Acetyl groups present in Vi Antigen. Important interactions related to its adsorption were observed through three-dimensional optimized structures. Two models were proposed for the Chitosan-Vi Antigen in adsorption system, one as a monomer and another as an optimized tetrasaccharide antigen. The Molecular Modeling studies presented the best conformation and binding site on the nanoparticle Chitosan-Vi Antigen in models proposed. Interactions were observed between *O*-Acetyl and *N*-Acetyl groups the Vi Antigen and hydroxy, amino and methyl groups the Chitosan.

1. Introduction

In developing regions (Asia, Africa, Central America and South America) typhoid fever is still a serious public health problem, because it leads to death more than 222 000 people every year [1,2]. According to the World Health Organization [3], it is estimated that typhoid fever affects about 21 million people annually, of which around 4% end in death [3,4]. Studies show that the incidence is higher in children aged below two years of age [3,5,6].

The agent responsible for the occurrence of typhoid fever is *Salmonella enterica* serovar Typhi, a gram negative bacillus belonging to the Enterobacteriaceae family, glucose fermenter and which has three antigens: O or somatic antigen, H or flagellar antigen and Vi or capsular antigen, which in turn are of great importance for the diagnosis of typhoid fever. These bacteria are uniquely adapted to mankind, who are their only natural host. They may be transmitted through raw or undercooked food, or even by drinking contaminated water [7–9].

The typhoid fever is an acute bacterial disease related to the endotoxin of the bacteria, with clinical manifestations such as abdominal pain, diarrhea, high fever, rash, papular erythematous lesions; it may reach more severe manifestations such as hepatosplenomegaly, intestinal bleeding and even more rarely, intestinal perforation and femoral thrombosis. In order to reduce transmission and ensure protection of individuals, the scientific community has been working on the development of vaccines containing Vi capsular antigen [7,8,10].

The Vi capsular antigen is a linear a homopolymer of (1 → 4) 2-deoxy-2-*N*-Acetyl galacturonic acid, where the C_3 group is variably *O*-Acetylated [11]. It is able to stimulate the production of antibodies, but the development of vaccines containing this antigen has complications because it is effective only during a short period of immunization, and is unprovided with immunological memory.

Thus, new strategies have been sought as the use of controlled release systems, exemplified by the conjugation of a carrier polysaccharide to a protein or other non-protein antigens. As

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an example of a controlled release system we can mention the nanocarriers, such as Chitosan nanoparticles which are able to encapsulate drugs, proteins or antigens, allowing this active agent to be gradually and slowly released [12]. Production of nanoparticles containing the antigen enables the stimulation of the immune system thereby generating a more durable immune response, and aiding in the protection against the bacteria [6,9,10,13,14]. The nanoparticles can be made from synthetic and/or natural polymers; in the latter kind Chitosan is an outstanding amino polysaccharide derived from the deacetylation process of the chitin considered a copolymer with 2-amino-2-deoxy-D-glucose and 2-acetamide-2-deoxy-D-glucose units, which are joined by glycosidic linkages of the type $\beta(1 \rightarrow 4)$ with its structure C_6 being composed of a primary amino group and two free hydroxyl groups [15,16].

Interest in controlled release systems has increased in recent years, and consequently, the search to improve this system. Computational Chemistry is emerging as an alternative for understanding the nanocapsules or nanospheres encapsulation, as well as their adsorption and release system [17]. The use of focused computer programs to study the chemical and networked databases are currently important tools for drug discovery and design [18]. These theoretical methods help in the identification and preparation of biologically active compounds through studies related to their structure, activity, metabolism, as well as their mechanism of action [19]. In Computational Chemistry, Molecular Modeling consists in creating theoretical models, in atomistic scale, which describe or interpret macroscopic properties that lead to improved absorption kinetics of a drug or the mechanism of action of a substrate [18,19]. Molecular Modeling through computer softwares makes it possible to study the physicochemical properties and the three-dimensional visualization of the molecular steroelectronic properties, as well as the elucidation of the interaction between drug and target macromolecules [19].

In this work, the Vi Antigen of *Salmonella* Typhi was extracted, purified and identified by Infrared Spectroscopy. The theoretical infrared spectra of the Vi Antigen was calculated and compared to the experimental. Additionally, the theoretical study was proposed to assess the intramolecular interactions between Chitosan and the Vi Antigen. The results of this study can be used as support for future development of vaccines against typhoid fever.

2. Experimental section

2.1. Materials and reagents

A strain of *Salmonella enterica* serotype Typhi was kindly provided by the Instituto Nacional de Controle de Qualidade em Saúde (INCQS) from the Oswaldo Cruz Foundation (FIOCRUZ). Sodium azide, hexadecyltrimethylammonium bromide, deoxyribonuclease and ribonuclease were supplied by Sigma Aldrich® (Germany). Tris (hydroxymethyl) aminomethane was acquired from Cientifica® Passos (São Paulo).

2.2. Obtaining strains

Lyophilized strains of *Salmonella* Typhi were resuspended in 1 mL of nutrient broth and streaked in *Salmonella-Shigella* Agar to its best growth. Grown bacteria were subjected to biochemical and serological tests to confirm the presence of Vi Antigen and then stored in Agar nutrient in the refrigerator for later use.

2.3. Vi capsular antigen extraction from Salmonella Typhi

Wong and Feeley technique [20] was adapted to perform the extraction using multiple reagents and multiple centrifugations. For a considerable bacterial mass, an isolated colony of the stored strains of *Salmonella* Typhi, was streaked in TSB broth, for an exponential growth and then transferred to Agar nutrient in a glass bottle, and left for 24 h. After that time the mass of bacteria was extracted from the flask and stored in 50 mL Falcon tubes. The bacterial cells were killed before the antigen isolation; the same technique employed by Wong et al. [21]. The antigen isolation began with the addition of 0.1% sodium azide solution in the killed cells, and stirred in water bath for 30 min and then centrifuged at 11,000 rpm for 30 min. Centrifugation finished, the supernatant was isolated and transferred to another Falcon tube.

To the supernatant, 1 mL of Tris-HCl buffer and 500 μL of ribonuclease and deoxyribonuclease enzymes were added and placed under stirring for 6 h at 37 °C. After this time 500 μL of pronase was added and stirred for 12 h. Subsequently, NaCl 5% (w/v) was added and the mixture was stowed in the freezer until freezing. Then 6 mL of pre-cooled ethanol was added and kept in the refrigerator overnight.

This mixture was centrifuged for 30 min at 11,000 rpm and then the pellet was isolated, and 5 mL of 60% ethanol in saline solution was added and stirred for 24 h. After this period, the supernatant was centrifuged and extracted. This process was performed twice more to yield a supernatant pool.

The supernatant was frozen at 2 °C and then an equal volume of pre-chilled ethanol was added and the mixture was kept in the refrigerator for 15 min. Subsequently, it was subjected to centrifugation of 11000 rpm at 2 °C for 30 min. The pellet was isolated and 10 mL of sterile 0.85% saline solution and 0.01 g of hexadecyltrimethylammonium bromide (w/v) were added and centrifuged at 8000 rpm for 15 min; 4 mL of 1 M KCl solution was added to the isolated sediment and then this mixture was filtered through 30.000 KDa ultra thin millipore filter. The tube containing the mixture was submitted to centrifugation of 7000 rpm for 15 min for an efficient filtration.

The recovery of the antigen retained on the filter was performed by the dropwise addition of 2 mL of ethanol PA and centrifuged at 8000 rpm for 20 min. The sediment which contained the antigen was removed and 5 mL of saline solution and 2 mL of ethanol PA were added, obtaining a suspension of the antigen. The suspension was lyophilized and stored for use in later techniques.

2.4. Infrared technique in Vi Antigen identification

Infrared spectroscopy in antigen identification is one of the most important and well applied analytical techniques. In this study, we used this technique of Fourier transforms to identify the characteristic functional groups of the Vi Antigen. The lyophilized sample obtained from the isolation and purification method, was sprayed with 99 mg of potassium bromide (KBr) and subjected to a high pressure to form the tablet. The spectra were obtained on a Shimadzu IRPrestige-21® spectrophotometer, in the wavelength range from 4000 to 400 cm^{-1} during 32 scans with a resolution of 4 cm^{-1}.

2.5. Computational session

In order to have a better understanding of the interaction between Chitosan and the Vi capsular antigen, a Molecular Modeling study of these structures was performed to comprehend the possible interactions between Chitosan and Vi Antigen during adsorption process and thus its stability. The structures were initially treated separately. The three-dimensional (3D) structure of the Chitosan was obtained from the NCBI (National Center for Biotechnology Information), which had the monomer and its molecular structure. As for the Vi Antigen we used the postulated model from the study by Yang et al. [22] using the monomer and tetrassacharide derived from the antigen, where the structure was generated using the ChemDraw Ultra 12 software (CambridgeSoft